

QUANTUM DOT / OPTICAL PROTEIN BIO-NANO HYBRID SYSTEM FOR BIOSENSING APPLICATIONS

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ABSTRACT

The unique energy transfer interaction between the optical protein bacteriorhodopsin (bR) and CdSe/ZnS quantum dots (QDs) provides a potential modulation mechanism for bio-nano electronic application. We have utilized ionic-self assembled monolayer (I-SAM) techniques to create a novel alternating monolayer system of QDs and bR on a conductive ITO substrate. Results demonstrate the ability to efficiently create bR/QD multilayer films along with the ability to control bR/QD spacing on the nanometer scale. I-SAM films of this nature demonstrate a sharp decrease in QD emission when deposited in close proximity to bR, suggesting possible fluorescence resonance energy transfer (FRET) effects in a bR/QD nanoscale system. The ability to modulate the QD photonic output based on proximity to bR in the I-SAM films could provide a direct method to modulate the electrical output for bio-nano sensing applications.

1. INTRODUCTION

The evolutionary development of biological systems has created an array of natural nano-scale materials with capabilities beyond that of current technology. Bacteriorhodopsin (bR) is one such material that has been intensely studied over the years due to its inherent ability to function as a light-driven proton pump (Haupts, Tittor et al. 1999). Structurally similar to the visual rhodopsin found in the mammalian eye, bR strongly absorbs visible light in the 570nm spectral region due to the attached retinal chromophore (vitamin A). With the absorbed photonic energy, the retinal undergoes a cis-trans isomerization and initiates a proton pumping mechanism that can be sensed via an electronic substrate. For engineered applications, the fact that bR is an integral membrane protein purified into cell membrane fragments known as purple membrane (PM), a crystalline structure is provided that allows the protein to remain functional over a wide range of environmental conditions (Hampp 2000)

In the present research efforts, the photoelectric properties of bR are utilized as the transducer substrate in

a bio-nano sensing device. The proposed sensing mechanism is achieved by harnessing the energy transfer interactions of bR and colloidal quantum dots (QDs) at the nano-scale. Previous efforts in our group have shown the ability for QDs to photonicly activate the bR photocycle on the macroscale (Griep, Cheam et al. 2006) along with suggesting a fluorescence resonance energy transfer (FRET) relationship in a bR/QD aqueous system (Griep, Winder et al. 2007). As shown in Figure 1, the QDs can be selected to allow for maximal overlap of the QD emission and bR absorbance peak.

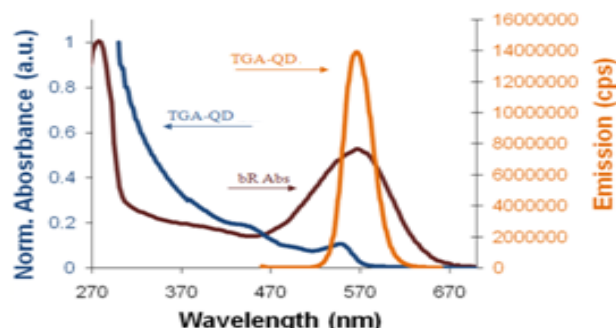


Fig. 1. Optical absorption and emission properties of size selected TGA-capped CdSe/ZnS QDs along with the absorbance spectra of bR.

To further study the bR/QD interactions in a dried state, particularly the potential FRET coupling relationship, nano-scale assembly techniques must be utilized as the FRET relationship is only achieved with sub-10nm bR/QD separation distances. In order to achieve close proximity along with a high degree of film orientation we have used Ionic Self Assembled Monolayers (I-SAMs) techniques which utilize the inherent charge on the material to build alternate positively/negatively charged monolayers through electrostatic adsorption. The utility of the I-SAM technique has been separately explored for both bR (He, Samuelson et al. 1997; He, Samuelson et al. 1999; He, Friedman et al. 2005) and QD (Constantine, Gattas-Asfura et al. 2003; Jaffar, Nam et al. 2004) thin film deposition. The results from He et al. in particular demonstrate that bR retains its functionality in these

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films, thus it is feasible to utilize the I-SAM method as a means of creating multi-layer bR/QD thin films for bio-nanoelectronic applications.

2. EXPERIMENTAL

The bR used in these studies was obtained from *H. Salinarium* strain S9P and prepared on-site according to standard methods. For the I-SAM work, the bR was suspended in Milli-Q distilled and deionized (ddI) water at pH 9.4 to facilitate a strong negative dipole within the protein (He, Samuelson et al. 1997). The QDs used in this study are CdSe(core) – ZnS(shell) capped with thioglycolic acid (TGA) to give the QDs a strong negative charge. The QDs were prepared according to previously published procedures and suspended in 0.05M NaOH (Zucolotto, Gattas-Asfura et al. 2005). The optical properties of the bR and TGA-capped QDs are shown in Figure 1. It can be noted that the size of the CdSe/ZnS QDs was specifically selected as to focus maximal photonic emission in the general 570nm absorption vicinity of bR. The bR and QD absorption spectra in Figure 1 are normalized and thus not quantitatively comparative, but bR/QD I-SAM studies suggest that each monolayer of TGA-capped CdSe/ZnS QDs had approximately twice the absorbance peak magnitude (at 545nm) than the bR 570nm absorbance peak (data not shown).

The ITO substrates (5x25x0.5mm) upon which the I-SAM films were constructed were cleaned using standard techniques and a negative surface charge was achieved by placing the substrates into a 2% KOH solution and agitating for 30 minutes in an ultrasonic bath. All ITO slides were stored in Milli-Q ddI water until use. Polydimethyldiallylammonium chloride (PDAC) was prepared to 2mg/ml in 0.5M NaCl pH 6.8 solution for use as the positively charged I-SAM. The positively charged PDAC served as a binding substrate material upon which the negatively charged bacteriorhodopsin and TGA-capped quantum dots could be electrostatically absorbed to.

The dipping mechanism of a Langmuir-Blodgett trough (KSV-2000) was used to dip the ITO substrate in a stable/consistent manner to the desired adsorbing material. The monolayers were assembled by submerging the ITO slide into the solution of the desired material for a set length of time: bR-5minutes, QDs-10minutes, PDAC-5minutes. Each adsorption period was followed by a thorough rinse in pH 9.4 Milli-Q ddI and the substrate was dried with nitrogen. Experiments were performed building bR/PDAC bilayers, QD/PDAC bilayers, and bR/PDAC/QD trilayers. The absorption/emission properties of the I-SAM multi-layered films were measured on a Perkin-Elmer Lambda 950 UV/VIS/NIR spectrometer and a Jobin Yvon Horiba Fluormax 3,

respectively. Topography measurements were performed with a Veeco CP-II atomic force microscope (AFM).

Measurements of the electrical activity of the bR I-SAM films were performed with a Keithley 4200 SMU. A test fixture was created to precisely control contact between the thin protein film and a top ITO electrode.

3. RESULTS AND CONCLUSIONS

For a baseline control, I-SAM films of both bR/PDAC bilayers and QD/PDAC bilayers were separately assembled. As shown in Figure 2, AFM images confirm the absorption of a single PM monolayer patches which corresponds to the published PM thickness of 5.5nm. Certain sections display membrane overlap with thicknesses around 11nm which has been observed in previous studies (He, Samuelson et al. 1997).

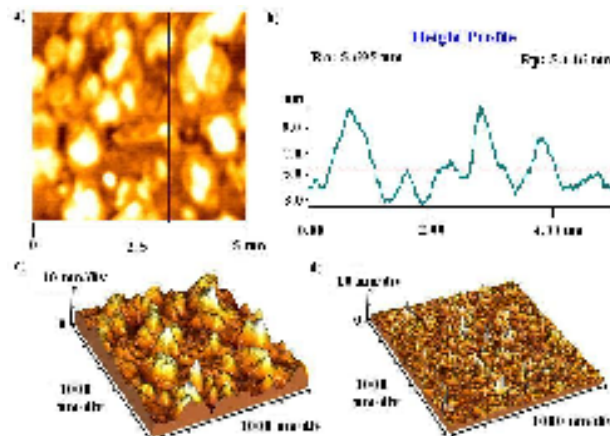


Fig. 2. AFM images of (a) I-SAM PM monolayer, (b) corresponding height profile showing the protein membrane fragments, and comparative 3D-topography of (c) I-SAM PM monolayer and (d) blank ITO.

The bR/PDAC bilayer assembly was monitored by the 570nm absorption peak of bR and demonstrated stable film deposition up to 12 bilayers as shown in Figure 3. The inset of Figure 3 shows the linear growth in bR absorbance corresponding to each deposited layer. The number of bR layers could theoretically be increased to create a thicker protein layer, however literature has shown that to achieve maximal photovoltaic output that a total of 6-8 bR monolayers be deposited (He, Samuelson et al. 1999). Maximal output is seen as lower monolayer thicknesses due to the measurement of the photovoltaic activity in a KCl electrolyte solution, thus the actual phenomena being measured is a pH change at the counter-electrode surface due to the bR proton pumping.

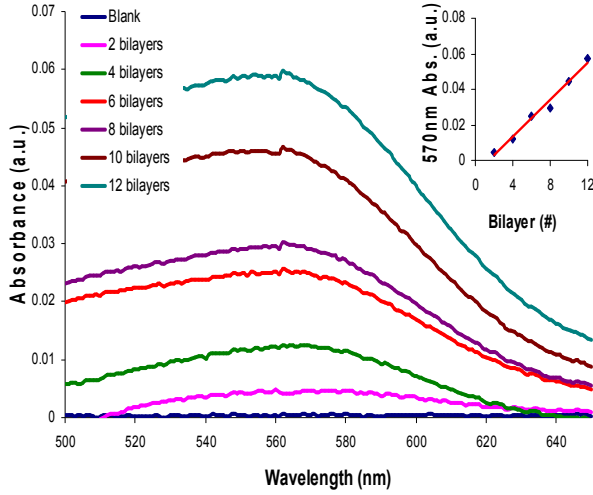


Fig 3. bR absorbance spectra for select bR/PDAC bilayers as it is assembled. Inset tracks 570nm absorbance of the bR retinal during consecutive bilayer depositions.

As shown in Figure 4, QD/PDAC bilayer films were created and show a linear increase in 570nm QD emission as each bilayer was assembled. This inset in Figure 4 tracks the 570nm QD emission for each deposited layer and demonstrates a linear increase on QD photonic output for each added layer.

With the results confirming that bR/PDAC and QD/PDAC bilayer I-SAM films could be separately constructed with the current method, emphasis was placed on the integration of bR and QDs into a multilayered I-SAM film. The layering structure of the first layer of this conjugate system, for example, is: ITO-PDAC-bR-PDAC-QD, with the pattern repeating (excluding the ITO) for consecutive layers. The bR/PDAC/QD conjugate film growth can be tracked by monitoring the increasing QD emission peak ($\lambda=570\text{nm}$), as shown in Figure 5.

Results show a linear increase in QD emission following each *full* trilayer deposition. Compared to the QD/PDAC bilayer I-SAM films, there was on average a 20% reduction in QD emission for each respective bR/PDAC/QD trilayer. The QD quenching effect is further exemplified by the *half* trilayer measurements, which correspond to the addition of PDAC/bR on top of the previous *full* trilayer. The inset in Figure 5 shows the quenching effect of the additional bR layer on top of the full trilayer, which results to an additional 20% reduction, on average, to the QD emission. The QD quenching effects can be attributed to a combination of bR absorption in the QD emission spectra along with potential fluorescence resonance energy transfer (FRET) between the QDs and bR retinal.

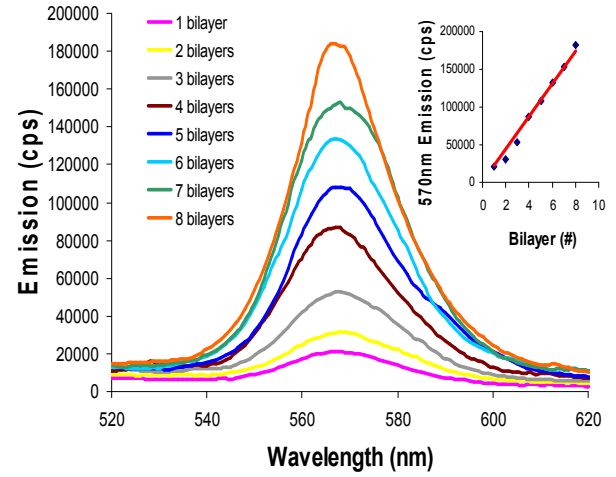


Fig 4. QD fluorescence emission for each QD/PDAC I-SAM bilayer as it is assembled. Inset displays linear trend in QD monolayer assembly.

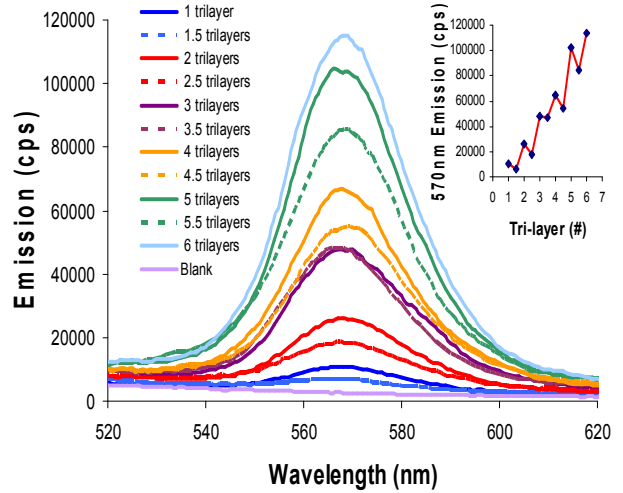


Fig 5. QD fluorescence emission for each bR/PDAC/QD I-SAM trilayer as it is assembled. Inset displays effects of bR on QD emission during tri-layer assembly.

The bR I-SAM films produced by the described techniques retain their photoelectric properties. As displayed in Figure 6, the photovoltaic response of a 12 bilayer bR/PDAC I-SAM film can be upwards of 50 mV. Photovoltaic signal from a bR substrate can be measured down to a single monolayer as displayed in Figure 7. The difference in signal shape in Figure 6 and Figure 7 is due to being AC and DC-coupled, respectively. The ability to achieve a significant bR electrical output at the monolayer level gives the opportunity to activate the bR photoactivity with the photonic output of integrated QD monolayers.

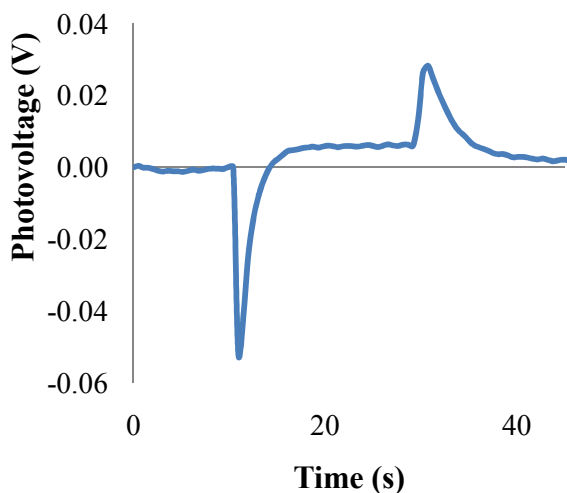


Fig. 7. Photoelectric response of a 12 bilayer bR/PDAC I-SAM film excited by a 50mW incident halogen light source. AC-coupled measurement mode.

To identify the effects of FRET and bR optical absorption, layers of bR were added to a single QD monolayer. As shown in Figure 8 and Figure 9, the first bR monolayer induced a 39% reduction in QD emission. All subsequent layers resulted in QD quenching of less than 10%. Since FRET is primarily observed when the acceptor (bR)/ donor (QD) pair are within 10nm, only the first bR layer would be within FRET proximity and thus resonance energy transfer is the probable cause for the additional quenching seen by the initial layer of purple membrane. Previous results (not shown) also demonstrate the bR/QD FRET phenomena in an aqueous system that corresponds to that observed in the dried film state.

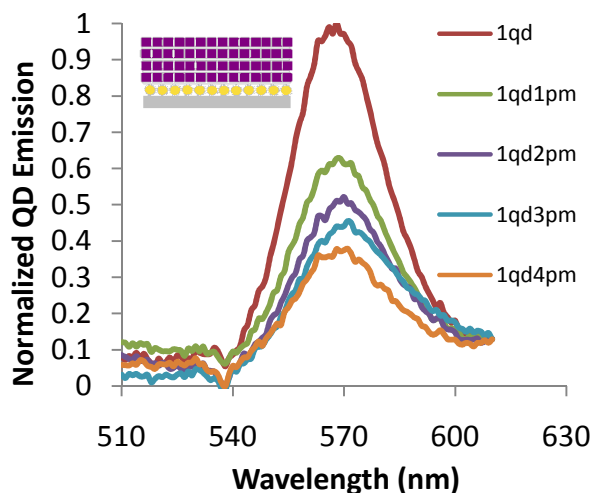


Fig. 8. QD fluorescence reduction with respect to added PM layers. The first PM layer resulted in a 39% reduction in QD emission intensity, while all subsequent layers induced less than 10% QD quenching.

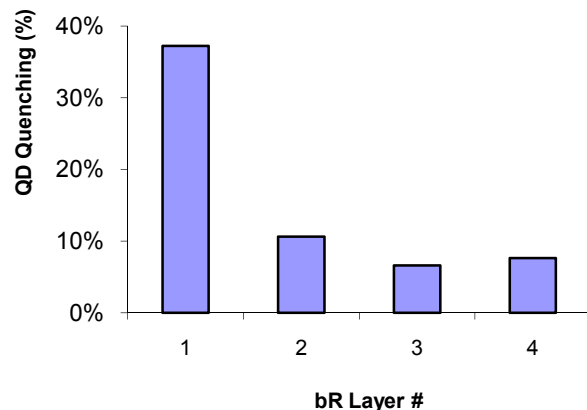


Fig. 9. Quenching effects of consecutive bR monolayers deposited on a single QD monolayer.

Future work will focus on verifying the FRET interactions along with characterizing the electrical output of bR/QD I-SAM electrodes, QD activation of bR monolayers, and demonstration of a maltose sensing prototype. In conclusion, this work verifies the ability to engineer nanoscale bR/QD multilayered films utilizing the I-SAM technique and allows further research to be performed on the nano-electronic properties of the bR/QD system.

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